CLAIMS

1. A method for preparing a DNA fragment corresponding to a nucleotide sequence of a 5' end region of an mRNA, comprising the steps of:

(a) preparing a nucleic acid corresponding to a nucleotide sequence of the 5' end of an mRNA;

(b) attaching at least one linker to the nucleic acid;

(c) cleaving the nucleic acid with a restriction enzyme having its recognition site within the linker and its cleavage site within the nucleic acid corresponding to the 5' end of the mRNA; and

(d) collecting a resulting DNA fragment corresponding to the 5' end of the mRNA.

2. The method according to claim 1, the length of the DNA fragment is about 5-100 bp.

3. The method according to claim 1, the length of the DNA fragment is about 15-30 bp.

4. The method according to claim 1, the length of the DNA fragment are about 10-30 bp.

5. The method according to claim 1, wherein the nucleic acid in step (a) is derived from one selected from the group consisting of a total RNA, an mRNA and a full-length cDNA.

6. The method according to claim 1, wherein step (a) comprises the steps of:
substituting a 5' cap structure of the mRNA with an oligonucleotide; and
synthesizing a first-strand cDNA using the mRNA as a template to produce a nucleic acid corresponding to the 5' end of the mRNA.

7. A method for preparing a DNA fragment corresponding to a nucleotide sequence of a 5' end region of an mRNA, comprising steps of:

(a) substituting a cap structure of an mRNA with an oligonucleotide, wherein the oligonucleotide comprises a restriction enzyme recognition site, and a cleavage site of a restriction enzyme is within the nucleic acid corresponding to the 5' end of the mRNA;

(b) synthesizing a first strand cDNA using the mRNA as a template;

(c) synthesizing a second strand cDNA using the first stand cDNA as a template;

(d) cleaving a resulting double stranded cDNA with the restriction enzyme; and

(e) collecting a resulting DNA fragment corresponding to 5' end of the mRNA.

8. The method according to claim 1 or 7, wherein the nucleic acid in step (a) is derived from a biological sample, an in vitro synthesized RNA, a cDNA library, artificially created pluralities of nucleic acids, or a tag library.

9. The method according to claim 1, wherein step (a) comprises the steps of:

synthesizing first-strand cDNAs using RNAs as a template and producing cDNA/RNA hybrids of the resulting first-strand cDNAs and the RNAs;

selecting a particular cDNA/RNA hybrid that has the 5' cap structure of the mRNA using a selective binding substance which specifically recognizes the 5' cap structure; and

recovering a nucleic acid corresponding to the 5' end of the mRNA.

10. The method according to claim 9, wherein the nucleic acid prepared in step (a) is a full-length cDNA, wherein the selective binding substance is attached to a support.

11. The method according to claim 1, wherein step (a) comprises the steps of:

synthesizing first strand cDNAs using RNAs as a template and producing cDNA/RNA hybrids of the resulting first strand cDNAs and the RNAs; and

recovering a nucleic acid corresponding to the 5' end region of the mRNA from the cDNA/RNA hybrids.

12. The method according to claim 1, wherein step (a) comprises the steps of:

synthesizing first strand cDNAs using RNAs as a template and producing cDNA/RNA hybrids of the resulting first strand cDNAs and the RNAs;

conjugating a selective binding substance to a 5' cap structure of an mRNA present in the RNAs;

contacting the cDNA/RNA hybrids with a support, wherein another matching selective binding substance is fixed to the support, and the matching selective binding substance specifically binds to the selective binding substance; and

recovering the a nucleic acid corresponding to the 5' end of the mRNA from the mRNA fixed to the support.

13. The method according to claim 9 or 10, wherein the selective binding substance is a cap binding protein or a cap binding antibody.

14. The method according to claim 12, wherein the selective binding substance is biotin, and the matching selective binding substance is selected from the group consisting of avidin, streptavidin and a derivative therefrom which specifically binds to biotin.

15. The method according to claim 12, wherein the selective binding substance is digoxigenin and the matching selective binding substance is an antibody against digoxigenin.

16. The method according to claim 10 or 12, wherein the support is made of magnetic beads, agarose beads, latex beads, sepharose matrix, silicagel matrix or glass beads.

17. The method according to claim 1, wherein step (b) comprises the steps of:

attaching a linker to an end region corresponding to the nucleotide sequence of a 5' end region of the mRNA, wherein the linker carries at least one restriction enzyme recognition site for a restriction enzyme that cleaves a site different from its recognition sequence;

synthesizing a second-strand cDNA using the nucleic acid as a template;

treating a resulting linker-bound double-stranded cDNA with the restriction enzyme; and

recovering a resulting fragment which contains a linker moiety and a part of cDNA corresponding to the 5' end regions of the mRNA.

18. The method according to claim 17, wherein the linker contains a double-stranded oligonucleotide region, and the second-strand cDNA is synthesized using the linker.

19. The method according to claim 17, wherein the second-strand cDNA is synthesized using other oligonucleotides which are partially or totally complement to the linker.

20. The method according to claim 17, wherein a selective binding substance is attached to or included in the linker, and the recovering step comprises the steps of binding the selective binding substance to a matching selective binding substance immobilized on a support, and recovering the support, wherein the matching selective binding substance specifically binds to the selective binding substance.

21. The method according to claim 20, wherein the selective binding substance is biotin, and the matching selective binding substance is selected from the group consisting of avidin, streptavidin and a derivative therefrom which specifically binds to biotin.

22. The method according to claim 20, wherein the selective binding substance is digoxigenin, and the matching selective binding substance is an antibody against digoxigenin.

23. The method according to claim 17, wherein the restriction enzyme is the Class II or Class III restriction enzyme.

24. The method according to claim 17, wherein the restriction enzyme is the Class IIG and Class IIS restriction enzymes.

25. The method according to claim 23, wherein the restriction enzyme is selected from the group consisting of Gsu I, MmeI, Bpm I, BsgI and EcoP15I.

26. A method for determining a nucleotide sequence of the 5' end region of the mRNA by sequencing the DNA fragment prepared by the method according to claim 1.

27. The method according to claim 1, further comprising amplifying the nucleic acid corresponding the 5' end region of the mRNA by a DNA polymerase or a cocktail of DNA polymerases.

28. The method according to claim 27, wherein the DNA polymerase is heat-stable.

29. The method according to claim 27, wherein the DNA polymerase is selected from the group consisting of Taq polymerase, Pwo DNA polymerase, Kod DNA polymerase, Pfu DNA polymerase, Vent DNA polymerase, Deep Vent DNA polymerase, rBST DNA polymerase, and Master Amp AmpliTherm DNA polymerase.

30. The method according to claim 1, wherein the first strand cDNA is synthesized and fractionated by physical means.

31. The method according to claim 30, wherein the nucleic acid is fractionated by hybridizing to a plurality of nucleic acids.

32. A method according to claim 1, further comprising the step of attaching the collected nucleic acid to beads.

33. A method for preparing a concatemer comprising one or more DNA fragments, comprising the step of ligating one or more of DNA fragments obtained by the method according to claim 1 and corresponding to the 5' end of the mRNA.

34. A concatemer prepared by the method according to claim 33.

35. A vector comprising the concatemer according to claim 34.

36. A sequence derived from the concatemer according to claim 34.

37. The method for determining the transcriptional states of a sample using a sequence derived from the DNA fragment prepared by the method according to claim 1.

38. The method for obtaining expression data on a plurality of mRNAs or cDNAs in a sample using a sequence derived from the DNA fragment prepared by the method according to claim 1.

39. The method quantifying expression data on a plurality of mRNAs in a sample using a sequence derived from the DNA fragment prepared by the method according to claim 1.

40. The method for building a database holding sequence information using a sequence derived from the DNA fragment prepared by the method according to claim 1.

41. The method identifying transcribed regions from a genomic sequence using a sequence derived from the DNA fragment prepared by the method according to claim 1.

42. The method for identifying a transcription initiation site and a related regulatory sequence in a genomic sequence using a sequence derived from the DNA fragment prepared by the method according to claim 1.

43. The method for cloning a full-length or partial cDNA from a cDNA library or biological sample using a sequence derived from the DNA fragment prepared by the method according to claim 1.

44. The method for cloning a complete or partial promoter region of a gene from a genomic library or genomic DNA using a sequence derived from the DNA fragment prepared by the method according to claim 1.

45. The method for analyzing the activity of regulatory regions in a genome based on genomic sequence information using a sequence derived from the DNA fragment prepared by the method according to claim 1.

46. The method for inactivating a gene or altering its expression using a sequence derived from the DNA fragment prepared by the method according to claim 1.

47. The method according to claim 46, wherein the gene is inactivated or altered in its expression by the means of siRNA or RNAi.

48. The method for synthesizing a nucleotide sequence to be used as the linker or primer based on a sequence derived from the DNA fragment prepared by the method according to claim 1.

49. The method for synthesizing a hybridization probe based on a sequence derived from the DNA fragment prepared by the method according to claim 1.

50. The method according to claim 49, wherein the hybridization probe is attached to a support.

51. The method according to claim 49, wherein the hybridization probe is a probe to identify the sequence corresponding to the nucleotide sequence of the 5' end region of the mRNA.

52. The method according to claim 1, further comprising extending the 5' end region of the nucleotide sequence.

53. A method according to claim 1 used for the development of diagnostic tools.

54. A method according to claim 1 used for the development of research tools.

55. A method according to claim 1 used for the development of a reagent or a kit.